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Novel small molecule bradykinin B_1 receptor antagonists. Part 2: 5-membered diaminoheterocycles

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ABSTRACT

Efforts to find new bradykinin B_1 receptor antagonists identified 2-aminobenzimidazole as a novel core. Subsequent transformation into five-membered diaminoheterocycle derivatives and their synthesis and SAR is described. This resulted in compounds with low nanomolar activity.

Bradykinin (BK) and kallidin (KD) are peptidic kinins which act on the B_2 receptor and mediate acute physiological actions of kinins on the cardiovascular, renal, nervous and immune system.[1] They are metabolized by carboxypeptidase N and M, which remove the carboxy-terminal arginine residue to generate des-Arg-9-BK (DABK) or des-Arg-10-kallidin (DAKD). DAKD is the only known natural agonist for the human bradykinin B_1 receptor and appears to be an important mediator of inflammation and pain in man.[2]

Whereas the bradykinin B_2 receptor is constitutively expressed under normal conditions in most cell types, the bradykinin B_1 receptor is induced under pathophysiological conditions such as infections, inflammatory diseases and traumatic tissue injury.[3] It has been shown that the bradykinin B_1 receptor is induced by various pro-inflammatory stimuli (e.g., lipopolysaccharide, Interleukin-1β) in several cell types including endothelial, smooth muscle, leukocytes and neurons.[4,5] Several studies have confirmed the presence of kininogens, kallikreins and kinin receptors in immune cells supporting an important role for kinins in immune response and inflammation.[6,7]

Recent evidence strongly suggests an involvement of the bradykinin B_1 receptor in various types of human disorders including inflammatory and neuropathic pain,[8] cardiovascular diseases[9] as well as inflammatory diseases such as rheumatoid arthritis,[10] inflammatory bowel disease[11] and psoriasis.[12] Therefore the bradykinin B_1 receptor seems to be particularly useful as a therapeutic target for the severe conditions associated with pathological inflammation and pain, both acute and chronic.[13]

Recently, the discovery of semicarbazide-based B_1 receptor antagonists, similar to **1**, has been reported.[14] To identify additional new lead series, modification of the semicarbazide moiety was investigated. The biaryl moiety was kept intact, as it represents a privileged scaffold in drug discovery.[15] Previous studies[16,17] have shown that a hydrogen bond acceptor in close proximity to the biaryl, such as the carbonyl group of the semicarbazide of **1,** is essential for achieving high potency at the B_1 receptor. These findings prompted investigation of the structure–activity relationship (SAR) of compounds represented by the general structure **2** (Fig. 1). The subsequent optimization to five-membered diaminoheterocycles, as represented by **3**, is reported herein.

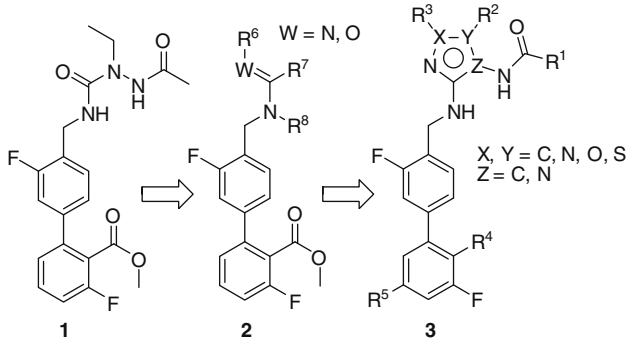

Figure 1. Strategy for the development of new B_1 receptor antagonists.

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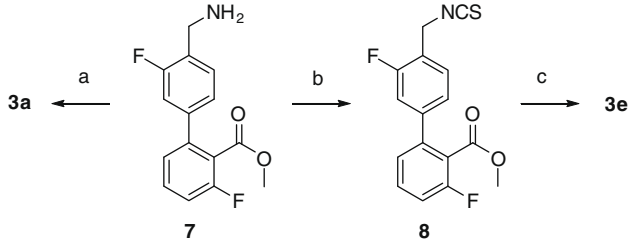
Different methods including reductive amination, alkylation, nucleophilic substitution and ring closing reactions were applied to install a hydrogen bond accepting functionality adjacent to the biaryl fragment resulting in compounds **3a–q** (Schemes 1–4).

Acetamide **3a** was obtained by standard acetylation of amine **7**[18] (Scheme 1). In addition, **7** was used to prepare the acetylated 1,2-diaminobenzimidazole **3e** via isothiocyanate **8** which was transformed with *N*′-(2-aminophenyl)acetohydrazide[19] to the corresponding thiourea which underwent intramolecular cyclization upon addition of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimid (EDC).

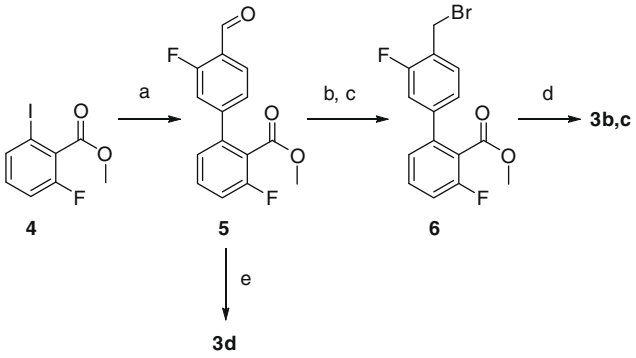
The synthesis of derivatives **3b–d** is shown in Scheme 2. The aldehyde **5** was prepared via Suzuki reaction from iodide **4** and 3-fluoro-4-formylphenylboronic acid. Compound **5** was either transformed to bromide **6** for alkylation reactions to yield **3b** and **3c** or directly used for reductive amination with 2-aminobenzimidazole to give **3d**.

For the synthesis of **3f** and **3l,** amine **9**[20] was converted to building block **10** utilizing the same reaction sequence as for the synthesis of **3e** (Scheme 3). The Boc protecting group of **10** was removed and the intermediate amine acylated to give the desired compounds.

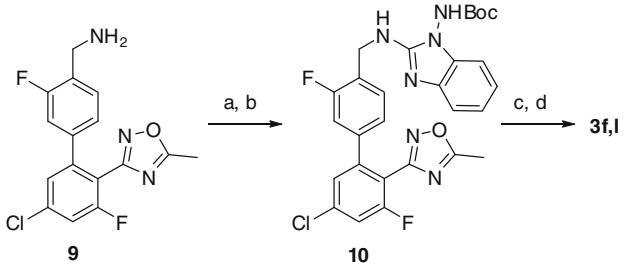
A set of 1,2-diaminoheterocycles were used as starting materials for the synthesis of compounds **3g–k** and **3m–q** (Scheme 4). The building blocks **11–14** were obtained from a three-component ring synthesis of an α-carbonyl chloride, a hydrazine amide and cyanamide. Intermediate **15** was obtained by chlorination of **11** with NCS. Intermediates **16**–**19** were synthesized in a one step acylation of commercially available starting materials (Scheme 4). Pyrazole **20** was prepared using standard transformation steps from commercially available 4-nitro-1*H*-pyrazol-3-amine. Building blocks **11**–**20** were obtained by reductive amination of aldehyde **22,** in turn synthesized from oxadiazole **21**[21] via Suzuki reaction (Scheme 4).



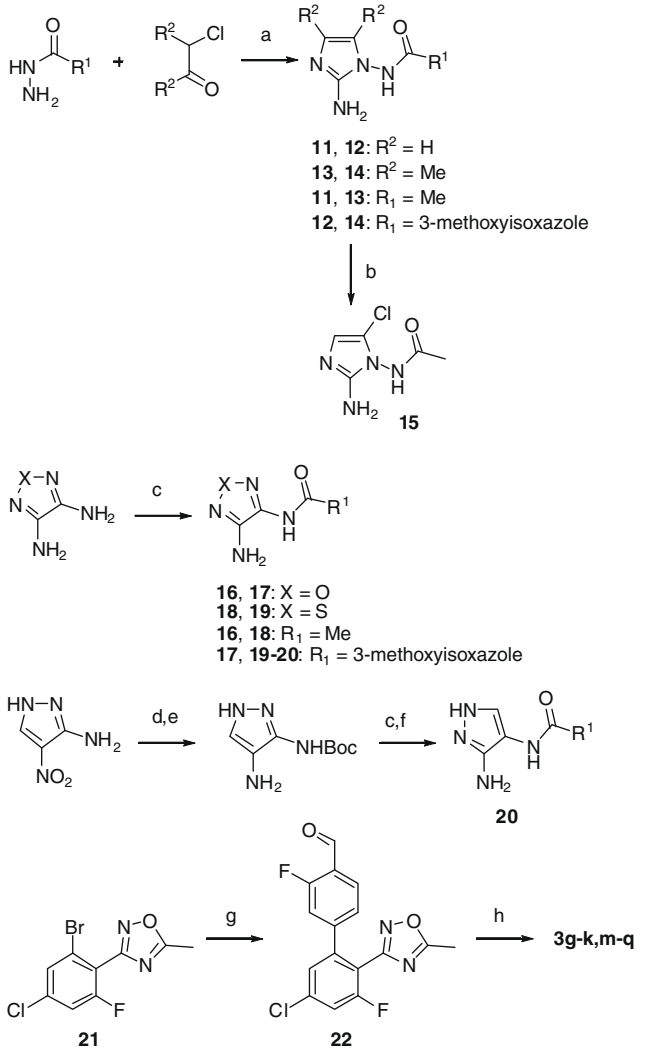
Scheme 1. Reagents and conditions: (a) Ac_2O; (b) $SCCl_2$, DCM/H_2O, $NaHCO_3$, 0 °C; (c) *N*′-(2-aminophenyl)acetohydrazide, DMF, rt, then EDC, 80 °C.



Scheme 2. Reagents and conditions: (a) 3-fluoro-4-formylphenylboronic acid, $PdCl_2(PPh_3)_2$, Na_2CO_3, 1,4-dioxane/H_2O, 80 °C; (b) $NaBH_4$, MeOH/DCM, rt; (c) PBr_3, DCM, 0 °C; (d) KHMDS, HNR′R″, NMP, rt or 60 °C; (e) 2-aminobenzimidazole, $Ti(O^iPr)_4$, dichloroethane, 60 °C, then $NaBH(OAc)_3$, rt.



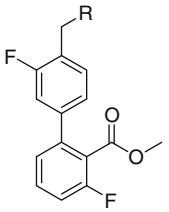
Scheme 3. Reagents and conditions: (a) $SCCl_2$, DCM/H_2O, $NaHCO_3$, 0 °C; (b) *tert*-butyl 2-(2-aminophenyl)hydrazinecarboxylate, DMF, rt, then EDC, 80 °C; (c) HCl, MeOH, rt; (d) 3-methoxyisoxazole-5-carbonyl chloride or Ac_2O, DIPEA, DCM, rt.

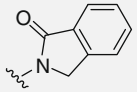
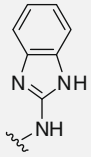


Scheme 4. Reagents and conditions: (a) H_2N–CN, AcOH, 60 °C; (b) **11**, N-chloro succinimide, DMF, 50 °C; (c) 3-methoxyisoxazole-5-carbonyl chloride or AcCl, THF, reflux; (d) Boc_2O, $NaHCO_3$, DMF, rt; (e) Raney-Ni, H_2, MeOH/ethyl acetate; (f) HCl, MeOH, rt; (g) 3-fluoro-4-formylphenylboronic acid, $PdCl_2(PPh_3)_2$, Na_2CO_3, 1,4-dioxane/H_2O, 80 °C; (h) **11**–**20**, $Ti(O^iPr)_4$, dichloroethane, 60 °C, then $NaBH(OAc)_3$, rt.

Table 1 shows the initial efforts to identify acetamide analogues of **1** endowed with reasonable activity. The in vitro functional activity at the B_1 receptor was assessed in a DAKD-mediated calcium mobilization (CAM) assay using human lung fibroblasts IMR-90 pretreated with IL-1 beta. Acetamide **3a** revealed a three orders of magnitude loss of potency when compared to semicarb-

Table 1
Antagonistic activity of **3a–d**

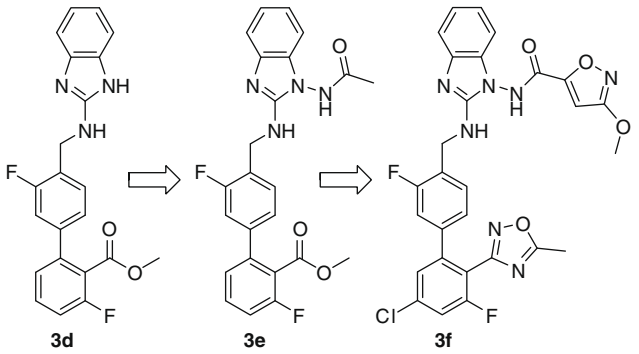


Compound	R	CAM IC_{50} (nM)[a]
3a		35,000
3b		42,000
3c		6100
3d		3500

[a] Numerical average of at least two experiments .

azide **1** (IC_{50} = 30 nM). Interestingly, the transformation of the acetamide group of **3a** to the hydrogen bond donor lacking pyrrolidinone **3b** had no substantial effect on the activity at the B_1 receptor, whereas the introduction of an annulated phenyl group increased the affinity by a factor of seven (**3c**). The most active compound in this series was found to be the benzimidazole derivative **3d**.

As the benzimidazole group represents a privileged scaffold in drug discovery, it was selected for further optimization. The transformation of **3d** to the optimized compound **3f** is depicted in Scheme 5. Adding an acetamide moiety to **3d** improved the IC_{50} value from IC_{50} = 3500 nM to IC_{50} = 15 nM (**3e**). Further optimization of the biaryl and the amide moieties led to compound **3f**. This compound exhibited excellent potency at the B_1 receptor (IC_{50} = 0.7 nM) but showed high P-gp efflux in a Caco-2 assay[22] (efflux ratio 7.1) and proved to be only moderately soluble (14 μM) in aqueous buffer. Recently, a similar six-membered diaminoheterocycle, that is, diaminopyridine,[23] was described as a scaffold for B_1 receptor antagonists. This 6-ring scaffold suffered from unwanted bioactivation, which led to irreversible protein binding.[24] It was proposed, that a 6-ring benzoquinone was involved as a reactive intermediate. In contrast, the five-membered heterocycles, disclosed herein, should be less prone to such a bioactivation because enzymatic oxidation is not facilitated by the formation of 6-ring benzoquinone-like resonance structures.[23] It will be a future task to verify this experimentally by metabolic studies.

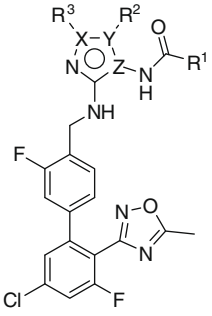


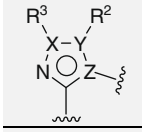
Scheme 5. Transformation of lead **3d** to the advanced lead **3e** and optimization to compound **3f**.

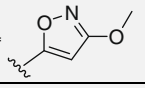
To increase the solubility, the benzimidazole core was optimized. Incorporation of imidazoles **3g** and **3h** resulted in much higher solubility in water (40 μM and 42 μM) than the benzimidazole **3f** (Table 2, left column). While the dimethyl derivative suffered from surprisingly poor activity, the removal of the methyl groups led to a significant increase in activity to IC_{50} = 1.3 nM (**3h**).

Alternative five-membered heteroaromatic core structures (**3i–k**) were also found to be highly potent. The most promising compound in this series was the furazane derivative **3j** which

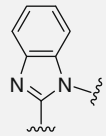
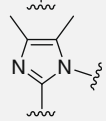
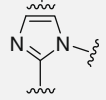
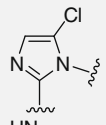
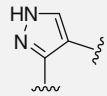
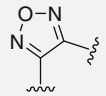
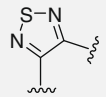
Table 2
Antagonistic activity of **3f–q**





R_1 = 

R^1 = Me

Compound	CAM IC_{50} (nM)[a]	Compound	CAM IC_{50} (nM)[a]
 **3f**	0.7	**3l**	4.6
 **3g**	85	**3m**	180
 **3h**	1.7	**3n**	1.6
 —	n.d.	**3o**	0.3
 **3i**	3.5	**—**	n.d.
 **3j**	1.0	**3p**	7.7
 **3k**	0.6	**3q**	1.7

[a] Numerical average of at least two experiments.

revealed good human microsomal stability (61% remaining after 1 h[25]), high permeability ($P_{app,\ A-B}$ = 48 × 10^{-6} cm/s) and no efflux in a Caco-2 assay (efflux ratio 1.0). Unfortunately, **3j** and **3k** were even less soluble than **3f**. To improve solubility, the isoxazole amide moiety was replaced by an acetamide (Table 2, right column). In the case of furazane (**3p**, IC_{50} = 7.7 nM) and thiofurazane (**3q**, IC_{50} = 1.7 nM) this was accompanied with a loss in potency although the expected increase in solubility was observed (e.g., **3p**, 21 μM vs **3j**, 12 μM). To further increase solubility, we again investigated the imidazole derivatives (**3l–o**). As with the isoxazole amide, the acetamide of the unsubstituted imidazole (**3n**, IC_{50} = 1.6 nM) led to a highly active B_1 receptor antagonist which was intolerant of dimethyl substitution with regards to activity (**3m**, IC_{50} = 180 nM). The most active compound was the chloroimidazole derivative **3l** which showed an excellent IC_{50} of 0.3 nM as well as good solubility in water (40 μM).

In summary, the discovery of a new series of B_1 receptor antagonists with five-membered heteroaromatic scaffolds is reported. Further optimization and pharmacological characterization of these compounds is ongoing.

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